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Interleukin-13: association with inflammation and cysteine proteolysis in varicose transformation of the vascular wall

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Abstract

The present review considers current data on the structure, functions and role of interleukin-13 in the pathogenesis of vascular wall varicose transformation in terms of proteolysis and inflammatory response. It is known that interleukin-13 is able to interact with transforming growth factor-β_1 in diseases associated with fibrosis. The latter activates fibroblasts and excessive formation of the extracellular matrix, thereby inducing fibrosis of the vascular wall, which is one of the links in the pathogenesis of varicose veins. Also, to date, there is evidence of the interleukin-13 participation in the induction of certain proteolytic enzymes' synthesis, such as matrix metalloproteinases. For the latter, participation in the transformation of the venous wall has been proven to date. The remodeling of the venous wall itself can lead to an increase in the expression of proteinases, providing a proteolytic mechanism for changing the structural organization of the venous wall in varicose veins of the lower extremities. At the same time, the involvement of lysosomal cysteine proteinases remains poorly understood. The expression and production of individual cathepsins are regulated by biologically active molecules: interleukin-1, interleukin-6, tumor necrosis factor α, which are directly involved in inflammatory reactions in the wall of varicose veins. In particular, venous pathology develops in a vicious circle of inflammation with the formation of abnormal venous blood flow, chronic venous hypertension and dilation, and the recruitment of leukocytes. This leads to a further, deeper, remodeling of the walls and valves of the veins, an increase in blood pressure and the release of pro-inflammatory mediators — chemokines and cytokines. In connection with the above, in order to understand the mechanisms of proteolysis in the vascular wall in varicose veins of the lower extremities, it is important to have an idea about the possible interactions of interleukin-13 with transforming growth factor-β_1, inflammatory cytokines, and cathepsins.

Keywords: interleukin-13, transforming growth factor-β_1, cathepsins B, H and L, varicose veins.

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Introduction

The involvement of cytokine series in the pathogenesis of various diseases remains a current research topic [1, 2]. Cytokines are soluble proteins with a molecular mass of 10–30 kDa that are produced by nearly all cells in the body. They serve as critical mediators that regulate immune and inflammatory reactions [3]. Cytokines are classified based on their biochemical and biological properties and receptor type. Depending on their role, cytokines can have either proinflammatory or anti-inflammatory effects [4]. Interleukins (ILs) are a group of cytokines that consist of 37 families, numbered from IL-1 to IL-37 [5]. IL-13 is one of the least studied members of this group [6].

IL-13 is a protein composed of 146 amino acids and has a molecular mass of approximately 13 kDa. The gene is located on human chromosome 5q31.1 [7]. Its conformation consists of four α-helices: A, B, C, and D [8]. IL-13 is primarily produced by activated Th2 cells, mast cells, basophils, eosinophils, NK cells, and mastocytes. IL-13 has two types of receptors, IL-13Rα_1 and IL-13Rα_2, each consisting of two types of subunits [9]. These receptors are expressed on various cell types, including endothelial cells, basophils, eosinophils, B-lymphocytes, fibroblasts, and mast cells, and they exert autocrine action. IL-13 receptors are also expressed in macrophages, monocytes, respiratory epithelial cells, and smooth muscle cells [6].

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IL-13, a cytokine of an inflammatory or anti-inflammatory nature

Currently, no consensus has been made on whether IL-13 has a proinflammatory or anti-inflammatory effect. M. Hussein et al. [10] demonstrated its proinflammatory effect in respiratory diseases. It plays a crucial role in inflammatory and fibrotic diseases, such as bronchial asthma, idiopathic pulmonary fibrosis, systemic sclerosis, and pulmonary granulomatous disease [10, 11].

N. Seyfizadeh [12] noted that IL-13 is a pleiotropic cytokine that acts through the functional IL-13Rα$_1$/IL-4Rα complex. This complex includes IL-13Rα$_1$, one of the IL-13 receptors, and a component of IL-4 receptors(IL-4Rα). This action is realized through Janus kinases and the activation of the transcription factor STAT6. IL-13 has various functions in fibroblasts, including inducing the expression of integrins, periostin, and proliferation, which contribute to the progression of inflammatory respiratory diseases, particularly bronchial asthma [13]. Binding to the second receptor, IL-13Rα$_2$, is a high-affinity interaction and acts as a negative regulator, although its mechanism is not fully understood [14].

Conversely, other authors believe that IL-13 plays an anti-inflammatory role in cardiovascular diseases. Ningjing Qian [15] reviewed the functions of IL-13 in cardiovascular diseases of different etiologies and concluded that IL-13 is involved in inflammatory heart diseases, such as myocarditis; as well as acute or chronic cardiovascular diseases, such as myocardial infarction and heart failure. IL-13Rα$_1$ and IL-13Rα$_2$ receptors are strongly expressed in cardiomyocytes, fibroblasts, vascular smooth cells, and endothelial cells in the heart [16]. However, the potential role of IL-13 in cardiovascular disease remains debatable.

S. O'Reilly [17] initially described IL-13 as a cytokine with an inhibitory effect on inflammatory cytokines. However, it is now recognized as a dominant cytokine that contributes to fibrosis [18].

Ramalingam et al. demonstrated that IL-13 plays a direct role in pulmonary fibrosis development. In addition, inhibiting IL-13 could reduce the severity of fibrosis and tissue remodeling. However, the mechanisms underlying these effects are not yet well understood [19].

Relationship between Il-13 and transforming growth factor-β$_1$ (TGF-β$_1$)

Studies have shown that IL-13 induces the production of TGF-β [13]. The relationship between IL-13 and TGF has been noted in the development of respiratory system pathology and fibrosis-associated diseases [20, 21]. TGF-β$_1$ is a multifunctional polypeptide that belongs to the TGF-β superfamily and consists of 112 amino acid residues [22]. It is an anti-inflammatory cytokine produced by T-helper cells (Th$_1$) [23].

TGF-β$_1$ is a crucial factor in embryonic development and physiological functioning of organs and systems. It is also involved in the pathophysiology of autoimmune, inflammatory, cardiovascular diseases, and fibrosis. TGF-β$_1$ is the primary isoform in cardiovascular pathology and is present in endothelial cells, vascular smooth muscle cells, myofibroblasts, and macrophages [22]. It also performs other essential functions. Specifically, it promotes extracellular matrix remodeling, stimulates fibrogenesis, and regulates the recruitment of leukocytes and fibroblasts [24].

Varicose veins have high TGF-β$_1$ levels in the vein wall. This protein is involved in the growth, development, and proliferation of smooth muscle cells and fibroblasts, which can lead to vein wall damage and varicose vein progression [23].

TGF-β$_1$ is involved in the remodeling of the extracellular matrix of the venous wall, which contributes to varicose vein development. It is crucially involved in the synthesis and degradation of extracellular matrix molecules such as collagen and proteoglycans [25]. In addition, it has been identified as a mediator of vascular fibrosis [22].

Under physiological conditions, fibrosis is a complex multistage process involving inflammatory cells, release of fibrogenic cytokines, growth factors, particularly TGF-β$_1$, and activation of collagen-producing cells [26]. However, in chronic cases, prolonged activation of myofibroblasts can lead to excessive and abnormal deposition of the extracellular matrix, resulting in fibrosis [22]. TGF-β$_1$ induces fibrosis by activating myofibroblasts, producing excessive extracellular matrix, and inhibiting extracellular matrix degradation [27]. Therefore, TGF-β$_1$ may contribute to the pathogenesis of varicose veins in the lower extremities [20].

Inflammatory nature of varicose veins in the lower extremities

Varicose veins of the lower extremities are a common form of cardiovascular disease [28, 29]. This condition can significantly affect patients' quality of life physiologically and socially [30], causing discomfort and cosmetic concerns [31]. The main symptoms include heaviness, soreness, itching, and burning in the lower extremities, which worsen with prolonged standing [32]. Varicose veins are a common reason for seeking healthcare, with an incidence rate of 25%–33% in women and 10%–20% in men [34].

Varicose vein pathogenesis is a topic of debate among researchers. Although some have claimed that inflammation is a contributing factor [35, 36], others refute this idea [37].

Varicose veins are mainly caused by venous hypertension, venous reflux, venous valve dysfunction, and venous wall inflammation [38–40].

Venous pathology can develop through a cycle of inflammation resulting in abnormal venous blood flow, chronic venous hypertension, and leukocyte involvement. This leads to vein wall and valve remodeling, increased intravascular pressure, and release of proinflammatory mediators such as chemokines and cytokines.

During the initial inflammatory process, endothelial permeability increases because of endothelial cell activation. Activated endothelium triggers adhesion through cell adhesion molecules such as selectins (E-, P-, and L-selectins), intercellular adhesion molecules, and vascular adhesion molecules. This leads to the migration of leukocytes through the vein wall, which release TGF-β_1 and proinflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor-α. This stimulates collagen synthesis by fibroblasts, causing vessel wall thickening and remodeling [38, 41].

Based on morphological studies, varicose veins can be classified into hypertrophic and atrophic. Hypertrophic varicose veins exhibit abnormal shapes and orientations of smooth muscle cells and accumulated extracellular matrix. In contrast, atrophic varicose veins demonstrate significant degradation of the extracellular matrix and tissue infiltration by inflammatory cells [42].

Because of increased venous pressure in the lower extremities, a cascade of events occurs. This includes damage to the vein endothelium and endothelial glycocalyx, increased endothelial cell permeability, activation of molecular adhesion, leukocyte infiltration into the vascular wall, and eventually vein inflammation. In varicose veins with severe inflammation, a structural change in the glycocalyx occurs.

Inflammatory response directly affects the composition of glucosaminoglycans, specifically certain combinations of disaccharides that make up different types of glucosaminoglycans, including heparan sulfate, chondroitin sulfate, dermatan sulfate, keratan sulfate, and hyaluronan. Consequently, the endothelial glycocalyx thins and loses its barrier function, and this may contribute to inflammation and vascular dysfunction because of the reduced rate and altered synthesis of glucosaminoglycans.

Leukocyte activation and expression of intercellular and vascular adhesion molecules are affected by changes in the venous tension shift and endothelial glycocalyx structure. The increase in hydrostatic pressure in the veins of the lower limbs, inflammation, and leukocytic infiltration of the vascular wall increase cytokine levels. These factors are the leading causes of progressive vein wall dilation in varicose veins [43, 44].

R. Solá Ldel demonstrated the role of inflammation in the physiopathology of chronic venous disease. The leukocyte "trapping" hypothesis suggests the link between venous hypertension and changes in macro and microcirculation. Leukocytes infiltrate the venous wall and valves, migrating through the endothelium of the postcapillary venules, leading to valve destruction and venous wall remodeling [45].

S.K. Tiwary et al. concluded that increased levels of the inflammatory marker C-reactive protein indicate endothelial damage in patients with varicose veins. In addition, blood obtained from the varicose vein site had significantly high levels of the proinflammatory cytokine IL-6, fibrinogen, and hemoglobin. This study confirms that inflammatory processes are activated in patients with varicose veins [46, 47].

In contrast, U. Sachdev indicated that patients with chronic venous insufficiency have lower levels of the inflammatory mediators IL-6 and tumor necrosis factor-α in their blood than the control group. This is consistent with hypoinflammation and a congestive post-inflammatory state [48].

According to Pfisterer et al., whether varicose veins are accompanied by venous wall inflammation remains unclear [49]. Macrophage accumulation was observed in both healthy and varicose veins, and proinflammatory adhesion was more pronounced in the later stages of varicose vein development. Recent publications suggest that inflammation may not necessarily be associated with varicose veins. Varicose veins may cause proinflammatory responses rather than the other way around [49].

Histological changes in the vascular wall of varicose veins

Varicose veins involve vascular wall remodeling, which can result in uneven thickness and significant thinning. Histological analysis by V.V. Studennikova revealed that fibromuscular rolls throughout the wall, with bundles of smooth muscle cells and collagen fibers perpendicular to the vessel wall. In addition, irregular hypertrophy of smooth muscle cells was found in wide areas of the walls. Many authors have emphasized the presence of fibrosis in the vascular wall of varicose veins. In their monograph, P.G. Shvalb and Y.I. Ukhov [51] described the micro-

scopic study of the vein wall in varicose veins. Wall changes comprise a different combination of rearrangement processes affecting all three vein sheaths. For instance, alterations in the intima are expressed as myoelastosis, myoelastofibrosis, sclerosis, and hyalinosis, as well as focal disorganization of connective tissue in the intima, such as mucoid swelling. Valve flaps often exhibit fibroelastosis with uniform or irregular thickening. Medial changes are marked by interfascicular fibrosis [51–53].

Barallobre-Barreiro et al. [54] and Studennikova [55] described the morphologic structure of varicose saphenous veins, noting extensive neointima formation, subendothelial fibrosis, wall thickening, and lumen dilation.

Pronounced subintimal and intermuscular fibrosis was also observed in the lower extremities.

Remodeling of the extracellular matrix architecture occurs in the vascular wall, leading to the destruction of the main stromal proteins, elastin and collagen. The regulation of damaged, fragmented elastin with disordered collagen distribution is disrupted [56]. This disruption causes increased levels of type IV collagen and decreased levels of type I collagen, resulting in impaired strength, flexibility, and structural integrity of the vascular wall [57].

The histopathological abnormalities of connective tissue components in varicose veins have been summarized. These abnormalities include disorganized arrangement of smooth muscle cells, extracellular matrix remodeling; weakening, fragmentation, and disruption of elastic fibers; and complete loss of the annular layered structure of collagen fibers, suggesting extensive proteolytic changes [56].

Varicose vein transformation disrupts the balance between enzyme synthesis activation and inhibition, resulting in the rapid turnover of collagen and elastic fibers [55]. Smooth muscle cells synthesize a significant amount of tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) while in an active state, which slows down the degradation of newly synthesized collagen fibers [58].

Cathepsins, a group of lysosomal proteinases, help in the remodeling of the extracellular matrix of blood vessels by degrading matrix components such as collagen and elastin and stimulating apoptotic processes [59]. In varicose veins, changes in the endothelium, extracellular matrix, and smooth muscle cells are key factors in vein wall remodeling [45].

Venous hypertension and/or wall hypoxia may activate the endothelium, endothelial and leukocyte adhesion molecules, and intercellular adhesion molecules, resulting in leukocyte activation and migration [22, 60]. This suggests that inflammatory processes may be involved in the vascular wall remodeling in varicose veins.

Proteolysis of the vascular wall of varicose veins: cathepsin involvement

Vascular wall remodeling in varicose veins of the lower extremities is accompanied by changes in proteolysis. Several classes of tissue proteolytic enzymes exist; however, we would like to focus on lysosomal cysteine proteinases [61].

Cathepsins (KF 3.4.22) are intracellular tissue enzymes located in lysosomes. They mainly possess endopeptidase effects and are involved in the cleavage of internal peptide bonds. Cathepsins perform an important regulatory function by breaking down proteins intracellularly. They also secrete and inactivate some enzymes, hormones, biologically active proteins, and peptides [62]. These enzymes have universal functions and are active in the low-pH environment of lysosomes [63].

Their activity increases in certain physiological conditions such as uterine involution after pregnancy, and in various pathological conditions, including muscular dystrophy, rheumatoid arthritis, gout, and pulmonary emphysema. This increase in activity is associated with the release of enzymes from lysosomes [64]. Cathepsins are enzymes found in nearly all animal tissues. Their activity is the highest in the liver, spleen, glandular tissue, and phagocytic cells such as macrophages and polymorphonuclear leukocytes. They are also highly active in rapidly growing and dividing cells [65].

Cathepsins are the major lysosomal proteases. They are classified into three main groups depending on their catalytic mechanisms: cysteine cathepsins (B, C, F, H, K, L, L, O, S, V, W, and X), serine cathepsins (A and G), and asparagine cathepsins (D and E) [66]. Serine proteases account for up to 31% of the total population of proteases in humans, cysteine proteases account for 25%, and asparagine proteases account for 4% of the total [67]. The relevance of cathepsin research remains high [68].

Cathepsin B (EC: 3.4.22.1) is a 30 kDa protein that consists of two distinct domains that interact through an extended polar interface, which opens the V-shaped cleft of the active site to the substrate. The protease has six disulfide bridges and two unpaired cysteine residues [69]. Cathepsin B is a cysteine peptidase belonging to the papain family that is expressed in all tissues. It plays a role in various physiological processes, including extracellular matrix remodeling (wound healing), apoptosis, and thyroxine and renin activation. In addition, cathepsin B is involved in several pathological processes, such as inflammation, parasitic infection, and cancer [70].

Cathepsin L (EC: 3.4.22.15), a single-domain monomeric protein with a mass of 25 kDa, is expressed ubiquitously and primarily localizes in lysosomes [71]. It is a cysteine protease that belongs to the cathepsin family. It can function both intracellularly and extracellularly, participating in processes such as extracellular matrix remodeling, protein degradation, apoptosis, autophagy, and immune responses. It also plays a significant role in various diseases, such as cardiovascular diseases, inflammatory diseases, diabetes mellitus, liver fibrosis, and cancer [72].

Cathepsin H (EC: 3.4.22.16) is a single-chain protein with a mass of 28 kDa (mature form). It is a cysteine protease that is ubiquitously distributed in cells and tissues [73]. When activated, cathepsin H can cleave a single N-terminal residue of a polypeptide chain as an aminopeptidase, but with lower efficiency as an endopeptidase [74]. Cathepsin H is a protease involved in intracellular protein degradation. Its expression is increased in pathological conditions such as prostate cancer, breast carcinoma, and melanoma [75]. In addition, it contributes to numerous diseases of the cardiovascular, bacterial, viral, parasitic, and neurodegenerative systems [76].

For a long time, cysteine cathepsins were considered proteases that were critical to nonspecific bulk proteolysis in the endolysosomal system [77]. However, a recent study showed that cathepsins play a crucial role in pathological processes of diseases such as cancer, rheumatoid arthritis, and various inflammatory diseases [78]. These lysosomal proteases are important factors in the development of cardiovascular diseases [79].

Recently, increasing evidence shows that cathepsins play an important role in various arterial diseases associated with atherosclerosis. However, their functional significance in varicose vein development remains unclear [80, 81].

However, N. Xu demonstrated the involvement of cysteine proteases in the pathogenesis of varicose veins [56]. Samples from varicose and normal human veins were studied using the immunohistochemical method. Xu reported that vein remodeling led to increased expression of cathepsins L, B, and K and decreased cystatin C in human varicose veins.

Vein remodeling is facilitated by an increase in the expression and activity of cathepsins, which provide a proteolytic mechanism. This process is associated with the activation of IL-1 and tumor necrosis factor-α, which regulate cathepsin expression in varicose veins. The increased proteolysis associated with inflammation can be confirmed by increased tryptase and chymase activity and abundance of mast cells and $CD3^+$ T cells, which serve as an important source of cathepsins. The activation of cathepsins and their inhibitors may contribute to vein remodeling through effective matrix degradation.

T cells in varicose veins contain a significant number of cysteinyl cathepsins. These proteases can remodel the extracellular matrix of the venous wall, infiltrate inflammatory cells, and cause remodeling and migration of smooth muscle cells because of their elastinolytic and collagenolytic activities [56, 82].

Conclusions

IL-13 may contribute to venous wall transformation by interacting with TGF-β_1, which activates fibroblasts and mediates vascular fibrosis. IL-13 induces fibrosis by stimulating myofibroblasts and promoting excessive synthesis of the extracellular matrix. This process disrupts vascular wall structure and function, initiating the progression of varicose veins. Vascular wall remodeling results in increased expression of cysteine cathepsins, providing a proteolytic mechanism for varicose vein damage involving inflammatory processes. L-13 and TGF-β_1 can be used as biomarkers for varicose veins in the lower extremity. This could positively affect the diagnostic and therapeutic options for this condition.

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